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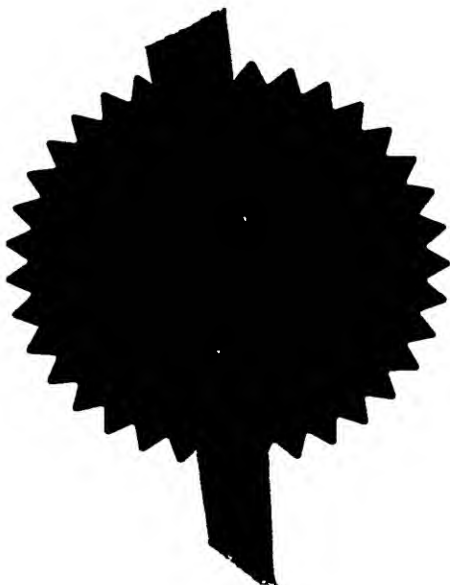
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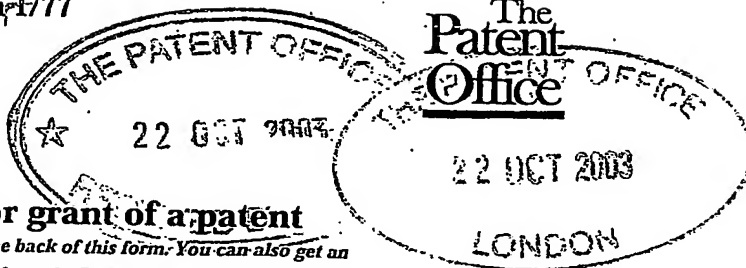
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22 OCT 2003

1. Your reference WJN/P36132GB

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0324641.0

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Unipath Limited
Priory Business Park
Bedford MK44 3UP

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

GB 06820989002

4. Title of the invention

Coagulation Detection Method

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Kilburn & Strode
20 Red Lion Street
London
WC1R 4PJ

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Number of earlier application

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Continuation sheets of this form

Description 12

Claim(s) 2 DL

Abstract

Drawing(s) 7+7

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Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*) 1 (to follow)

Request for preliminary examination and search (*Patents Form 9/77*)

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11. I/We request the grant of a patent on the basis of this application.

Signature W J Neobard

Date 20.10.03

12. Name and daytime telephone number of person to contact in the United Kingdom
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Coagulation Detection Method

5 The present invention relates to a method of detecting coagulation of a fluid and to apparatus constructed and arranged to detect coagulation of a fluid. The fluid may be blood.

10 Many devices have been proposed for determining the coagulation time of fluids, especially blood. Most of these have disadvantages for example high cost, or difficulty in obtaining reproducible results. In the field of blood coagulation measurements, it is desirable to use a small quantity of blood for the comfort of the patient.

15 According to one aspect of the invention there is provided a method of detecting coagulation of a fluid comprising providing a magnetic field to cause particles to move within the fluid, illuminating the fluid and optically detecting at least one of presence of the particles at a predetermined location in the fluid and movement of the particles through a predetermined location in the fluid. In one embodiment the magnetic field is such as to cause the particles to translate within the fluid.

20 According to another aspect of the invention there is provided a method of detecting coagulation of a fluid comprising providing a magnetic field to cause particles to move to and fro within the fluid, illuminating the fluid and optically detecting at least one of presence of the particles at a predetermined location in the fluid and movement of the particles through a predetermined location in the fluid.

25

According to a further aspect of the invention there is provided a method of detecting coagulation of a fluid comprising providing a container holding said fluid, applying a magnetic field at one zone of a container whereby particles move towards said one zone through the fluid, applying a magnetic field at
5 another zone of said container whereby said particles move through said fluid towards said another zone, illuminating the fluid and optically detecting at least one of presence of the particles at a predetermined location in the fluid and movement of the particles through a predetermined location in the fluid.

10 In an embodiment said one zone is one end of the container and said another zone is a substantially opposite end of the container.

In an embodiment the particles are paramagnetic.

15 In an embodiment the particles are superparamagnetic

In an embodiment there is provided at least one electromagnetic, wherein the method includes selectively controlling current applied to the or each electromagnet.

20 According to yet a further aspect of the invention there is provided apparatus constructed and arranged to detect coagulation of a fluid comprising a controllable magnetic arrangement operable to provide a magnetic field such as to cause particles to move within the fluid, a light source operable to illuminate the fluid and an optical detector operable to detect at least one of presence of
25 the particles at a predetermined location in the fluid and movement of the particles through a predetermined location in the fluid.

Embodiments of the invention provide a way to determine the coagulation time of blood using a low cost illuminator / detector pair to interrogate a blood sample for the presence or absence of particles that move under the influence of a magnetic field.

5

In some embodiments optical measurement of the particles takes place when the particles are stationary; in others the measurements occur when the particles are moving. Where stationary measurements are used, the output of the detector, or the input from the illuminators, or both may be gated in synchronism with spaces between the current pulses providing the magnetic pulses to cause particle movement.

10

15

In yet other embodiments optical measurement in the measuring zone is carried out regularly, say every 5-10 mS, and the noise level is assessed. Coagulation will have occurred when the noise changes abruptly or falls to zero.

20

In still further embodiments, the signal from the sensor is gated to open a window at a set time after an electromagnet is actuated. The period is selected according to the position chosen as the measuring zone so that movement of the particles through the measuring zone (if the movement is able to occur) is in fact occurring. Then the noise is compared from one window to the next to determine when coagulation takes place.

25

Exemplary embodiments of the invention will now be described with reference to the accompanying drawings, in which:

Figure 1 shows a block schematic drawing of part of first apparatus embodying the invention;

Figure 2 shows a block schematic drawing of part of second apparatus embodying the invention;

5 Figure 3 shows a block schematic drawing of part of third apparatus embodying the invention;

Figure 4 shows a glass capillary being used to demonstrate a method embodying the invention;

10 Figure 5 shows an exploded diagram of one embodiment of a test chamber for use in method and apparatus embodying the invention;

Figure 6 shows a perspective view of the assembled test chamber of Figure 5; and

Figure 7 shows a cross-sectional view of a chamber useable in the invention.

15 Referring to Figure 1, a pair of relatively low power electromagnets (4,5) is arranged exterior to a measurement container (7) which defines a chamber (3) in which blood coagulation is to be measured. The magnets (4,5) are arranged spatially such that when current to the magnets is correctly switched movement of magnetised particles disposed between the magnets (4,5) occurs between one
20 electromagnet and the other. The chamber (3), when in the dry state, in this embodiment contains particles (not shown) that move under the influence of a magnetic field, as well as reagents (not shown) that promote the coagulation of blood. The particles in this presently described embodiment are
25 superparamagnetic particles. The particles become suspended in solution upon contact with the blood sample and traverse the chamber as the electromagnets are alternately switched on and off.

An illuminator, such as an LED (14), is provided at a position exterior to the measurement chamber and positioned to illuminate a portion of the liquid contained within it. Either reflected or transmitted light may be measured using a detector (12) that is also positioned exterior to the chamber (appropriate baffling may be required to prevent direct input from the illuminator into the detector).

In the embodiment shown in Figure 1, the diode (14) is positioned such that the particles are illuminated whilst moving. In another embodiment as shown in Figure 2, a similar diode (114) and detector (112) are positioned such that the particles are illuminated when stationary. In this second embodiment, particles are moved to one electromagnet, which is subsequently switched off. At this point, the particles are illuminated. The polarity of the electromagnets is then reversed and the particles then move across the chamber. More than one diode may be employed, for example, one positioned at either end of the chamber.

In some embodiments the illuminator (14, 114) is selected to have a wavelength that has low reflection from the blood but high reflection from the particles; in others the opposite arrangement applies. In yet other embodiments the illuminator (14, 114) is time-modulated to remove ambient light and other electromagnetic effects.

Measuring the presence or absence of particles from a portion of the chamber is therefore relatively straightforward due to the direct timing control exercised over the electromagnets. The particle movement is monitored until some time that the change in signal is detected indicating coagulation or a change in viscosity.

A first-off illuminator/detector pair has been tested with blood and liquid research particles in a Camlab capillary. The hardware was un-optimised in respect to wavelength, modulation, gain, isolation and placement tolerance. A steady and repeatable 2mv signal change is detected readily using this set-up.

The container (7) in which the sample is held can be various designs and shapes.

- 10 In one embodiment of the invention the chamber is thin and flat in profile having dimensions where the top surface to be interrogated is 1-10 mm in each dimension and the chamber having a thickness in the range 10-500 μm . More preferable the chamber has a top surface to be interrogated of 1 mm x 2 mm and is 100 μm in thickness. Figure 1 depicts the top view of a container (7) defining a flat thin chamber (3) that has an inlet (1) and an exit (2) channel. Within the chamber there are detection zones (6) that can be interrogated for the presence or absence of particles. The particles are moved into and out of the field of view of the detection zones via electromagnets (4 and 5).
- 15
- 20 In the embodiment of the invention shown in Figure 3 the chamber is cylindrical in nature having dimensions where the top surface has an area of 0.25-10 mm^2 and a length of 0.5-2 mm. One preferred embodiment of the chamber has a top surface area of 0.5 mm^2 and a length of 1.6 mm. Referring to Figure 3 a cylindrical chamber has a chamber body (7) defining a chamber (3) with an inlet channel (1) and an exit channel (2). The chamber (3) has detection zones located towards the top and bottom surfaces of the chamber (6). The detection zones are interrogated for the presence or absence of particles. The
- 25

particles are moved via electromagnets (4 and 5). The inlet and out let channel can be along the same plane as shown in Figure 3. In other embodiments they are located such that one channel is in close proximity to the top surface and one channel is in close proximity to the bottom surface.

5

Examples

Detection of particles in a flat thin chamber

10 Super paramagnetic particles [Liquids research, cat number SC(2)] were mixed into 2 ml of sucrose at 3 % (w/v). An aliquot of particles (5 μ l) was mixed with 20 μ l of fresh venous whole blood. The blood containing particles was pipetted into a glass capillary (Camlab laboratory products, Cambridge, UK cat number VD/3520-100) and the glass capillary was inserted between two
15 electromagnets (RS, cat number 3305213).

Figure 4 shows a glass capillary having external dimensions of 2.4 mm width, 50 mm in length and 600 μ m thickness (internal dimensions of 2 mm width and 200 μ m thickness) inserted between two electromagnets (201 and 202). The
20 glass capillary has open ends and so have an inlet (203) and an air-venting exit (204). The electromagnets were driven by a simple electrical circuit that passed current at 60 mA into one electromagnet (201) for a duration of 250 ms and then switched the 60 mA current into a second electromagnet (202) for a duration of 250 ms, this was then repeated a number of times. As can be seen
25 in Figure 4a when the electromagnet (201) has current passing through it the super paramagnetic particles are located in a region (205) close to the electromagnet (201). By comparison in Figure 4b when the electromagnet ,

(202) has current passing through it the super paramagnetic particles are no longer within this region (206). It is possible to detect the presence or absence of particles within region (205) either using a simple camera system or using changes in light intensity from the surface.

5

Thromboplastin (InnovinTM, Dade Behring) and super paramagnetic particles can be mixed with fresh whole blood and the sample placed in the glass capillary. The electromagnets can be turned on and off and the presence or absence of particles can be determined as described above. The prothrombin time of coagulation can be determined by a change in the periodicity of the particles appearing and moving out of the detection zone.

10

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The profile of the alignment of the super paramagnetic particles within the generated magnetic field can be as "fingers" (207, 208 and 209) or as one mass (not shown) depending on the particle type and magnetic field generated.

Manufacturing methods

20

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A test chamber may be made using a relatively simple multi-layer (four layers or more) laminate construction to provide a low blood volume test device. Referring to Figures 5 and 6, in one embodiment a test device (100) is constructed from four layers (numbered 101 to 104 from top to bottom) of mylar type materials with thickness in the range from 100um to 200um each to provide an overall device thickness of around 600-800um for stability. Layers (101) and (104) are made of a material with low contact angle or treated on the underside and topside respectively to promote flow characteristics. Layers (101, 102 and 103) have features cut to the full depth of the material. Layer (101) has simple squares cut to match the sample application feature in layers (102) and

(103). Layer (102) has an adhesive coating on the top surface whilst layer (103) has adhesive coatings on both sides. In some embodiments adhesive coatings have hydrophilic properties. In some embodiments the bottom of layer (102) is treated to provide enhanced flow characteristics into the detection chambers.

5 Layer (102) contains a sample application feature, channelling to transport the blood sample to the corners of the detection chambers (two in the described embodiment) and venting channels/features at the opposite corners of the detection chambers. Layer (103) contains the same sample application feature as layer (102) and the detection chambers (2mm by 1mm).

10

Construction: The adhesive protector on the topside of layer (102) is removed and layer (102) is adhered to layer (101) with simple alignment of the squares in layer one with the sample application features in layer (102). The adhesive protector on the bottom side of layer (103) is removed and layer (103) is
15 adhered to layer (104) with no alignment. The reagents and particles that move in a magnetic field are dosed into the detection chambers. The adhesive protector on the topside of layer (103) is removed and the two sub-assemblies are adhered to each other using the sample application feature and detection chambers as alignment guides. The construction process is envisaged to take
20 place on a sheet basis initially where additional handling guides could be built into the layers and eventually on a web manufacturing process. The test strips once formed on a sheet or web basis would then be cut out as individual parts.

25

Other embodiments include more than two detection chambers, by the use of a separation layer between layers (102) and (103). In some embodiments layer (102) has adhesive on both sides. The separation layer then does not require any adhesive layers. Additional detection chambers are introduced in layer (103)

with fluidic separation from the channelling in layer (102). Another embodiment includes mini-wells for the deposition of reagents and particles through the use of an additional layer with adhesive on the underside between layers (103) and (104). This layer has smaller through holes (e.g. 2 per detection chamber) to coincide with the detection chamber holes in layer (103).

Figure 5 shows the four layers described in the first embodiment with the parts cut out as individual parts - layer (101) therefore does not have squares cut in the area of the sample application feature in layer (102) (these are lost). Figure 6 shows the assembled device.

This provides a low cost manufacturing method and design for a multiple detection chamber measurement strip with low sample volume (sub micro-litre) for the measurement of blood coagulation.

Manufacturing constraints are also eased by providing a simple laminate based, multi-layer (four or more), construction system with in-built fluidic separation to the required level for the detection of blood coagulation.

This also may provide major flow surfaces that are free of adhesive coatings. The sample inlet into the chamber, being via the top layer, is not affected by the deposition of particles and reagents.

Detection of particles in a cylindrical chamber

Super paramagnetic particles (Polymer laboratory) were mixed into 2 ml of sucrose at 3 % (w/v). An aliquot of particles (60 nl) was deposited into an

injection moulded plastic chamber (prepared using conventional injection moulding techniques). The contents of the plastic chamber were dried by subjecting the plastic part to infra-red radiation using a halogen short wave infra-red bulb (Philips Lighting, RS250-1050) producing a surface temperature of 55 °C for 3 minutes). The plastic part was covered with a hydrophobic laminate (3M, cat number 9795) and then inserted between two electromagnets. The apparatus was heated to 37°C by placing in a thermostatically controlled chamber. Fresh venous whole blood was pipetted onto the plastic part and allowed to migrate by capillary action into the chamber.

Figure 7 shows a cross section of the plastic chamber. The chamber is shown outlined (301) and has dimensions of approximately 0.5mm² top surface area, 0.3mm² bottom surface area and a depth of 1.6 mm. When the electromagnet located above the chamber (not show) has current applied the particles migrate towards the top of the chamber (302) and when the electromagnet located below the chamber (not shown) has current applied the particles migrate towards the bottom of the chamber (303). It is possible to detect the presence or absence of particles within the chamber either using a simple camera system or using changes in light intensity from either a side on view as seen in Figure 8 or from the top and/or bottom surfaces of the chamber.

Using the optical approach of the present invention, it is possible to create embodiments where sample volume in the chamber is reduced to 200nL. Close location of the optics is not necessary and particles can be moved in the x/y plane allowing a shallow detection chamber.

Use of a shallow chamber also allows for dosing of reagents into the normal 'dosing' plane (e.g. x/y) and also reduces the overall depth of the detection chamber and the blood volume required (sub-micro-litre) to run the test.

- 5 A double-sided design is used to allow channelling on one side of the device and detection chambers on the reverse side of the device. Due to the design the detection chambers can be shallow in the z dimension (around 100um deep) thereby reducing the blood volume required considerably whilst being large in the x and y directions (around 1mm by 2mm) thereby easing constraints on
- 10 particle and reagent dosing.

The device also eases constraints on blood sample filling due to the detection chamber itself being of capillary dimensions and the feed channel / detection chamber interface being at the natural corner of the detection chamber.

CLAIMS

1. A method of detecting coagulation of a fluid comprising providing a magnetic field to cause particles to move within the fluid, illuminating the fluid and optically detecting at least one of presence of the particles at a predetermined location in the fluid and movement of the particles through a predetermined location in the fluid.
5
2. A method as claimed in claim 1, wherein the magnetic field is such as to cause the particles to translate within the fluid.
10
3. A method of detecting coagulation of a fluid comprising providing a magnetic field to cause particles to move to and fro within the fluid, illuminating the fluid and optically detecting at least one of presence of the particles at a predetermined location in the fluid and movement of the particles through a predetermined location in the fluid.
15
4. A method of detecting coagulation of a fluid comprising providing a container holding said fluid, applying a magnetic field at one zone of a container whereby particles move towards said one zone through the fluid, applying a magnetic field at another zone of said container whereby said particles move through said fluid towards said another zone, illuminating the fluid and optically detecting at least one of presence of the particles at a predetermined location in the fluid and movement of the particles through a predetermined location in the fluid.
20
25

5. A method according to claim 4 wherein said one zone is one end of the container and said another zone is a substantially opposite end of the container.

6. A method as claimed in any preceding claim wherein the particles are paramagnetic.

7. A method as claimed in any of claims 1-5 wherein the particles are superparamagnetic.

8. A method as claimed in any preceding claim wherein there is provided at least one electromagnetic, wherein the method includes selectively controlling current applied to the or each electromagnet.

9. Apparatus constructed and arranged to detect coagulation of a fluid comprising a controllable magnetic arrangement operable to provide a magnetic field such as to cause particles to move within the fluid, illuminating the fluid and optically detecting at least one of presence of the particles at a predetermined location in the fluid and movement of the particles through a predetermined location in the fluid.

10. Apparatus according to claim 7 wherein the fluid is blood.

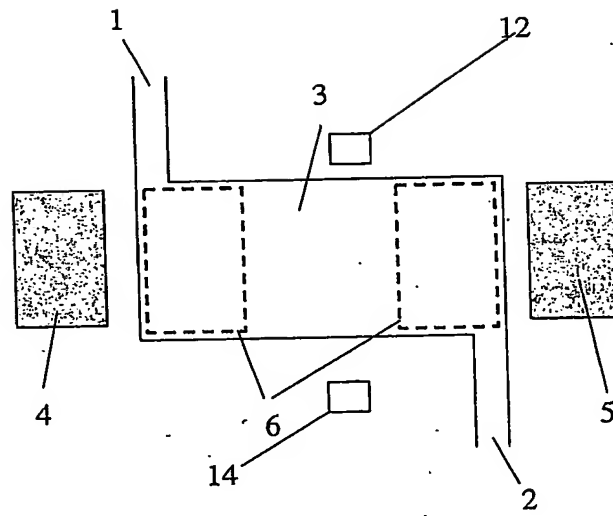


Figure 1

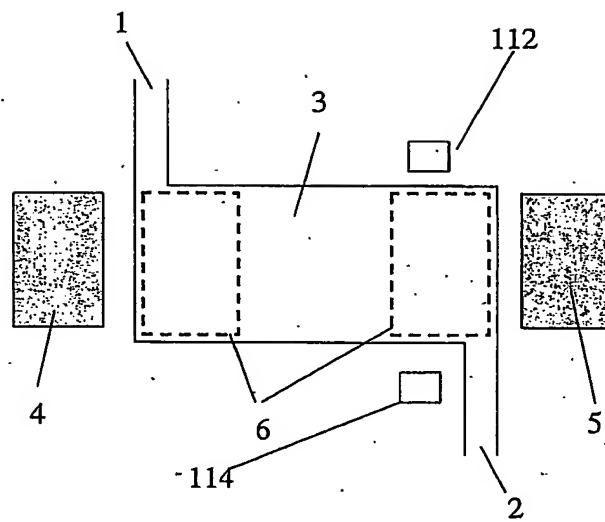


Figure 2

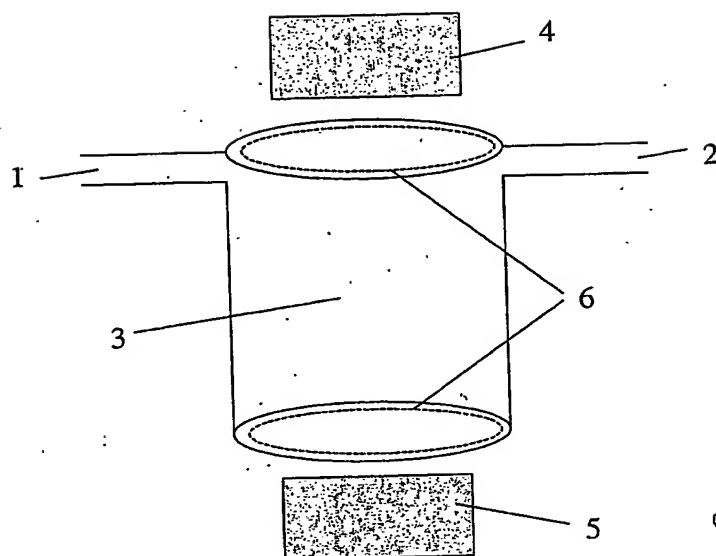


Figure 3

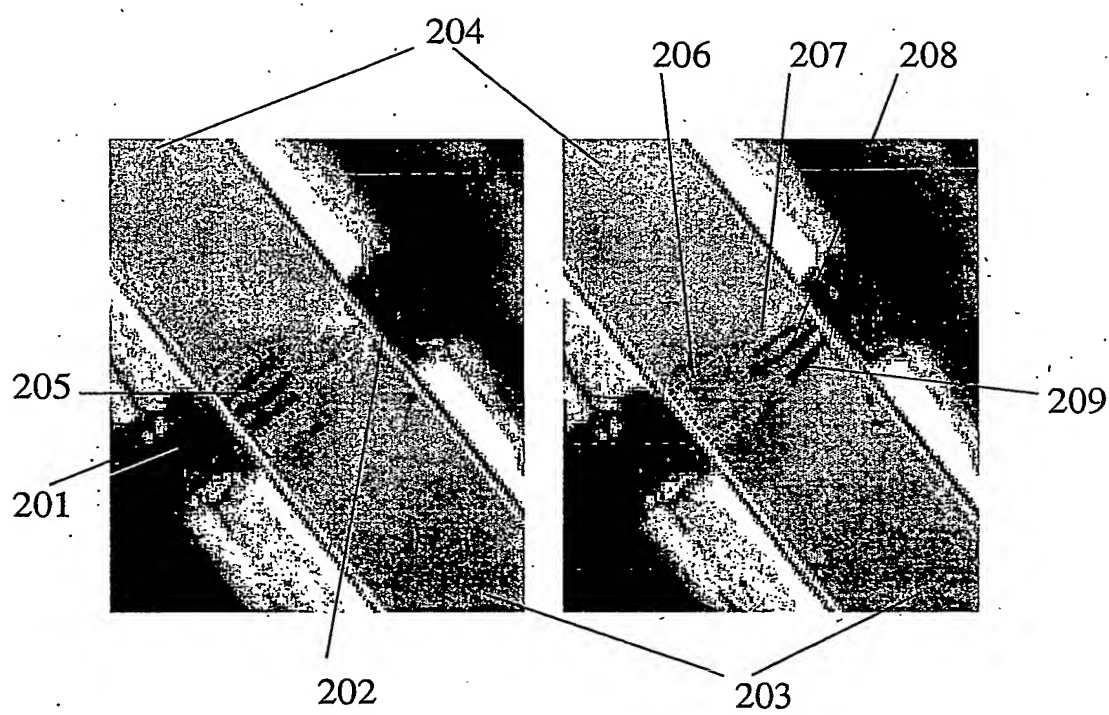


Figure 4

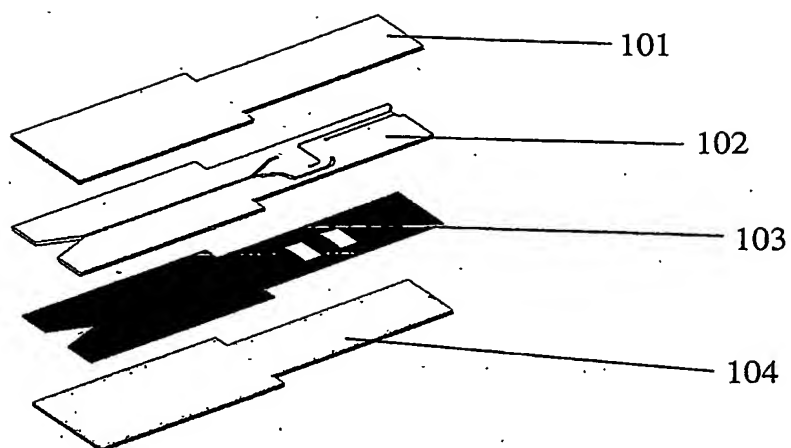


Figure 5

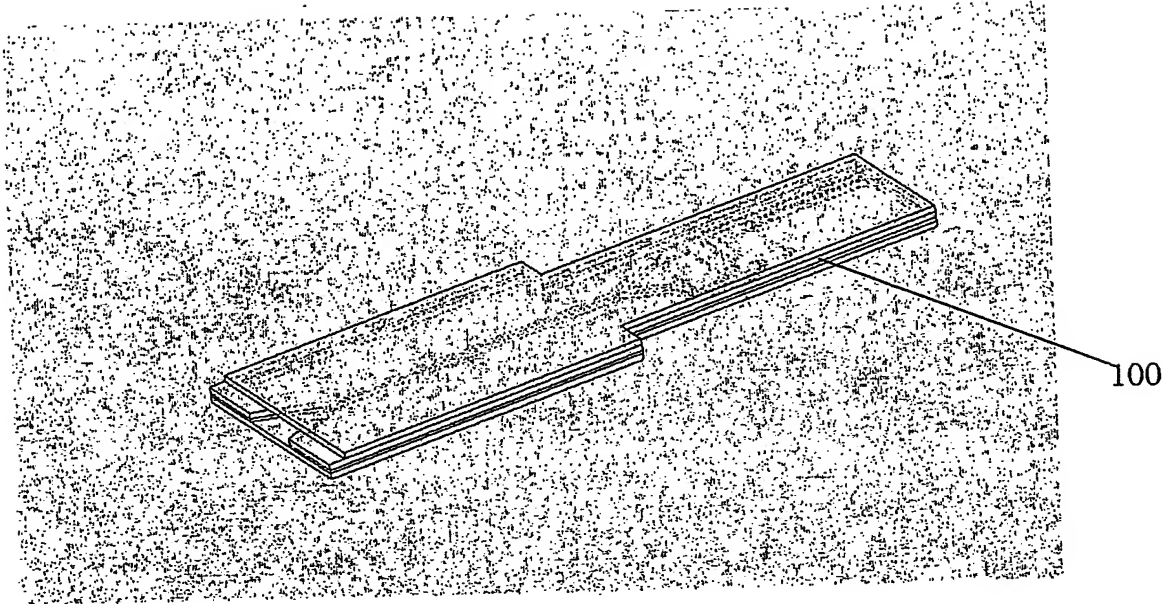


Figure 6

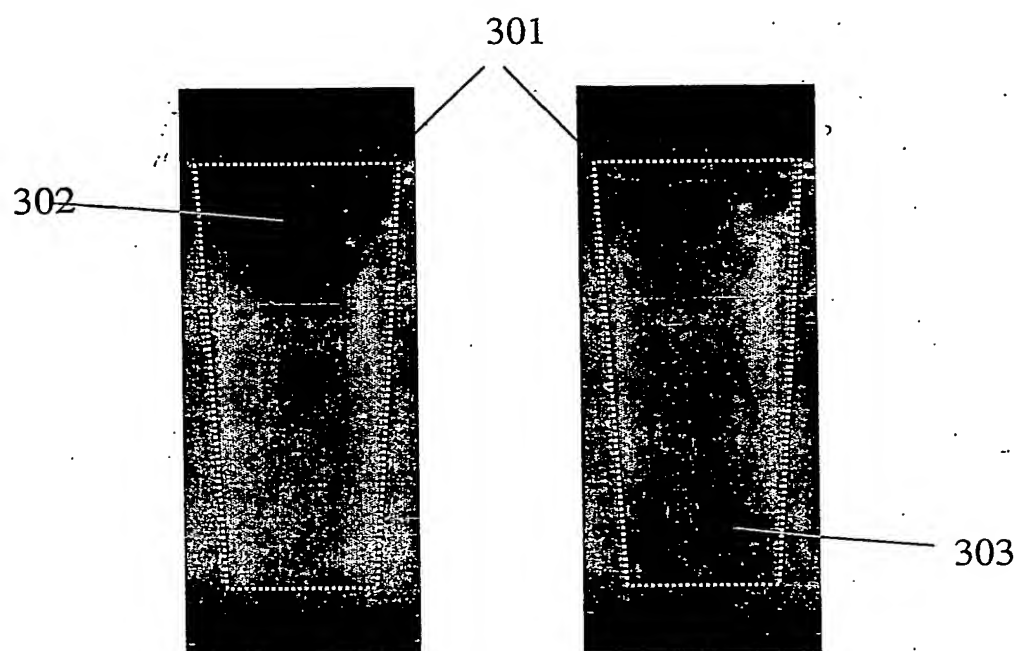


Figure 7